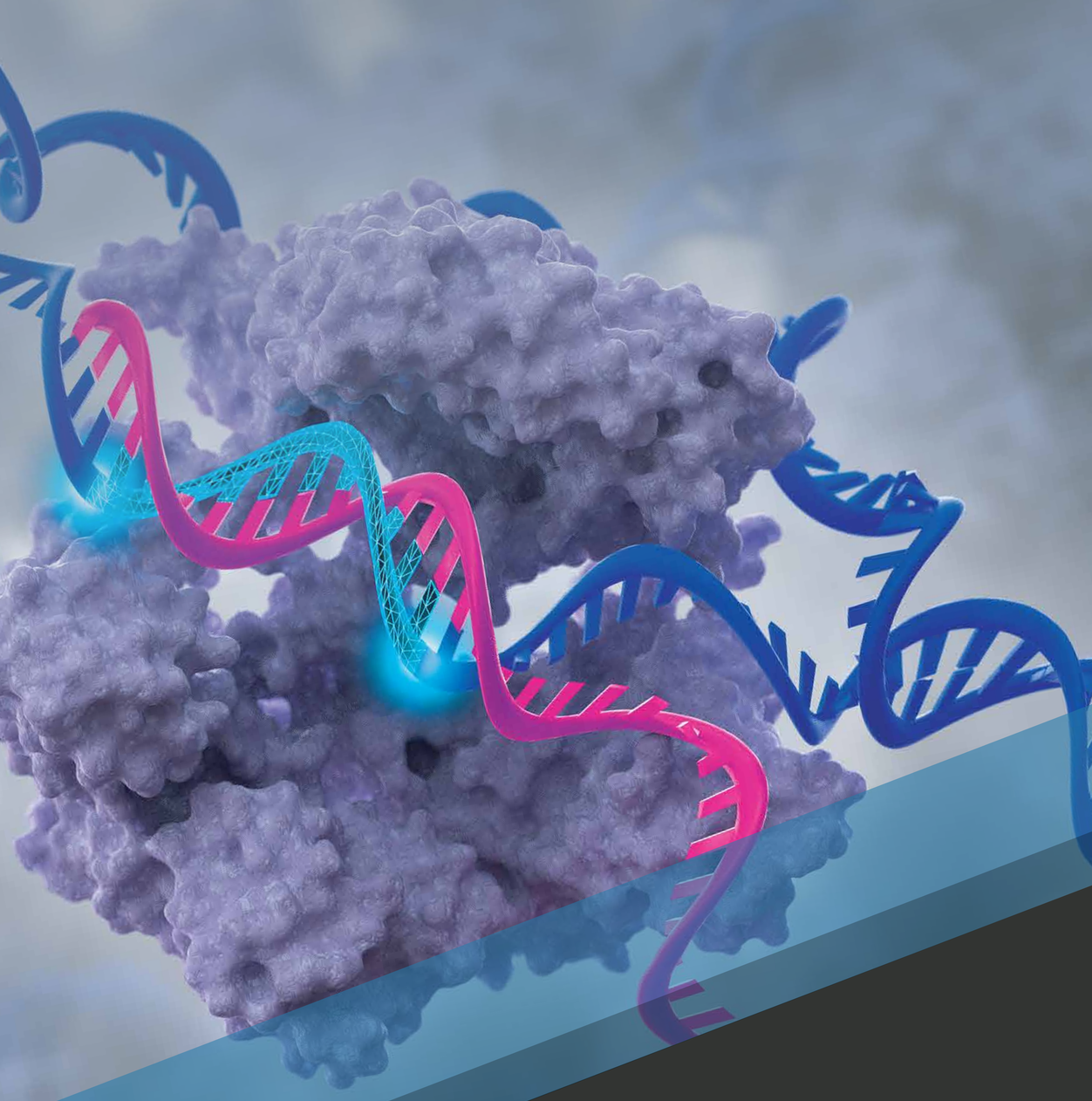
Cutting Edge science symposium

Genome Engineering for Cancer Treatment

Frank and Bobby Fenner Conference



http://genome-engineering.com.au

Canberra
20-22 Nov 2017

Welcome address

Alan Finkel
(Chief Scientist, Australia)

Keynotes

Laura Attardi
(Stanford, USA)

Danilo Maddalo
(Novartis Oncoloty, CHE)

Veera Baladandayuthapani
(MD Anderson, USA)

Organizers

Denis Bauer, CSIRO
Gaetan Burgio, ANU
Marco Herold, WEHI
Kaylene Simpson, PeterMac
James Doecke, CSIRO
Mark Tizard, CSIRO
Tim Doran, CSIRO
Laurence Wilson, CSIRO

Sponsors

CSIRO
Australian National University
millennium science — enabling next-generation research™
GENESEARCH eFREEZER
MERCK
agrf
Lonza
ThermoFisher SCIENTIFIC
BioTools — Innovative Tools for Life Sciences
apn
scientifix
addgene — The nonprofit plasmid repository
Walter+Eliza Hall Institute of Medical Research — DISCOVERIES FOR HUMANITY

Smarter, swifter, safer: facing cancer in the CRISPR age

Alan Finkel

Australia's Chief Scientist Dr Alan Finkel looks to the ten year horizon for genomics in Australian healthcare, premised on a human-first approach.

Targeted epigenetic editing for reprograming of complex phenotypes in breast cancer

Pilar Blancafort[1,2], Charlene Babra Waryah[1], Colette Moses[1,2], Jessica Kretzmann[3], Marck Norret[3], and Killugudi Swaminatha Iyer[3]

[1]Cancer Epigenetics, The Harry Perkins Institute of Medical Research
[2]School of Human Sciences, The University of Western Australia.
[3]School of Molecular Sciences, The University of Western Australia

The completion of genome, epigenome and transcriptome mapping in multiple cell types has created a demand for precision biomolecular tools that allow researchers to functionally manipulate DNA, reconfigure chromatin structure and ultimately reshape gene expression patterns. Here we will describe the generation of epigenetic editing tools to interrogate the relationship between epigenetic modifications and gene expression, and to functionally map regulatory regions such as promoters and enhancers, for both coding genes and small non-coding RNAs. Importantly, the reversible characteristics of epigenetic modifications offer an attractive therapeutic opportunity to reprogram complex phenotypes, such as metastatic behavior, via targeted epigenome engineering.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins adapted for epigenetic editing provides an unprecedented tool to regulate multiple genes and reprogram cell phenotypes. In this system catalytically defective dCas9 is fused to epigenetic modifying domains to target specific epigenetic marks to specific sites in the genome. Epigenetic editing of multiple tumour suppressor genes and oncogenes will be discussed, particularly for the reprograming of complex yet plastic and reversible gene programs such as epithelial-to-mesenchymal transition (EMT) in breast cancer. Finally, we will describe the development of novel tumour-specific delivery systems for CRISPR in mouse tumour models and outline potential applications for the future treatment of metastatic breast cancers for which no cure is available.

Deconstructing the p53 Tumor Suppression Program Through Combined RNA interference and CRISPR Screening

Kathryn T. Bieging-Rolett[1], Alyssa Kaiser[1*], David W. Morgens[2*], Jose Seoane[2,3,4], Stephano S. Mello[1], Jacob McClendon[1], Ian P. Winters[2], Christina Curtis[2,3,4], Monte M. Winslow[2,4], Michael C. Bassik[2] and Laura D. Attardi[1,2,4]

[1]Division of Radiation and Cancer Biology, Department of Radiation Oncology, [2]Department of Genetics, [3]Department of Medicine, and [4]Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA 94305
*These two contributed equally.

Despite the critical role of the p53 transcription factor as a tumor suppressor, the pathways through which p53 acts remain enigmatic. To better understand the transcriptional networks through which p53 suppresses cancer, we utilized both RNA interference and CRISPR/Cas9 screening approaches to query the importance of a set of p53 tumor suppression associated target genes that we had previously identified using mouse knock-in mutant models. As each platform has distinct on-target potency and off-target spectra, we reasoned that a combined approach would be the most powerful strategy to understanding p53-mediated tumor suppression. Interestingly, these screens have revealed common and distinct hits, emphasizing the value of both approaches. I will describe these screening experiments and how they have informed us on the mechanisms underlying p53 function in both mouse and human cancers.

Rapid, complex models of colorectal cancer: using CRISPR/Cas9 genome editing to target frequently altered genes.

Tamsin RM Lannagan[1], Young K Lee[1], Tongtong Wang[1], Jatin Roper[2], Mark L Bettington[3,4], Lochlan Fennell[4], Laura Vrbanac[1], Lisa Jonavicius[5], Roshini Somashekar[1], Krystyna Gieniec[1], Miao Yang[1], Jia Q Ng[1], Nobumi Suzuki[1], Mari Ichinose[1], Josephine A Wright[1], Rhonda Hutchinson[6], Wirapati P[7], Matthew Hussey[5], Tracy L Putoczki[8], Sonja Klebe[5], Sabine Tejpar[9], Barbara A Leggett[4], Vicki L Whitehall[4], Daniel L Worthley[1]*, Susan L Woods[1]*.

[1]School of Medicine, University of Adelaide and South Australian Health and Medical Research Institute, Adelaide, SA Australia
[2]Tufts Medical Center & Tufts University, School of Medicine, Boston, MA USA
[3]Envoi Specialist Pathologists, Brisbane, QLD Australia
[4]QIMR Berghofer Medical Research Institute, Brisbane, QLD Australia
[5]Department of Anatomical Pathology, Flinders Medical Centre, Bedford Park, SA Australia [6] Genetics and Molecular Pathology, SA Pathology, North Adelaide, SA Australia
[7] Swiss Institute of Bioinformatics, Bioinformatics Core Facility, Lausanne, Switzerland.
[8]Department of Medical Biology, University of Melbourne and the Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC Australia
[9]Digestive Oncology Unit, Department of Oncology, University Hospitals Leuven, Leuven, Belgium.
*=contributed equally

In this age of next generation sequencing we are fast accruing more information on cancer associated genetic alterations than ever before. How do we translate this new knowledge into better outcomes for cancer patients? Clearly we must prioritise genetic alterations for study from this wealth of data. Here we utilise the organoid culture technique, combined with CRISPR/Cas9 genome engineering, to sequentially introduce genetic alterations associated with the serrated pathway to colorectal cancer (CRC). This pathway accounts for 25% of colorectal cancer, including those with the worst prognosis. At the molecular level it is most commonly associated with activating mutations in the mitogen activated kinase (MAPK) pathway gene, *BRAF*, and epigenetic modifications termed the CpG Island Methylator Phenotype (CIMP). We have produced a series of organoids with alterations to genes in the MAPK, TGF, Wnt and senescence pathways that are associated with the serrated pathway using CRISPR technology. Orthotopic injection of the more complex organoid lines quickly generates CRC *in vivo* with serrated features reminiscent of the human disease. The penetrance of this phenotype and effect on survival is reduced when organoids with less genetic alterations are used. In this way we are rapidly producing novel, preclinical models of serrated colorectal cancer that can be readily personalised to investigate the many leads generated by next generation sequencing. We are using these models for preclinical therapeutic evaluation in known, complex genetic landscapes. We are also developing stool based, high sensitivity DNA tests for serrated CRC, to better detect these hidden lesions.

Whole genome in vivo CRISPR/Cas9 screens identify novel tumour suppressor genes in Eμ-Myc lymphomagenesis.

Shinsuke Mizutani, Ana Janic, Lin Tai, Andrew Kueh, Martin Pal, Andreas Strasser, and Marco J Herold

The Walter and Eliza Hall Institute of Medical Research

The transcription factor p53 has a crucial role in repressing tumour development but cellular processes that are critical in this process remain unclear. A full understanding of the mechanisms of p53 mediated tumour suppression is expected to give a better comprehension of tumorigenesis. This in turn should facilitate the development of improved strategies for the prevention, early detection and even therapy of cancer. One approach to achieve this is to identify novel tumour suppressors and then study their relationship with p53. Here, we performed an in vivo whole mouse genome sgRNA library screen using Eμ-Myc_Cas9 haematopietic stem/progenitor cells (HSPCs)to identify novel tumour suppressors. The mouse sgRNA library (Koike-Yusa et al. 2014) contains 87,897 individual sgRNAs targeting 19,150 murine protein-coding genes. The sgRNA library transduced Eμ-Myc_Cas9 HSPCs were transplanted into lethally irradiated wild type recipients to reconstitute their haematopietic system, and then aged to wait for development of B cell lymphoma. The sgRNAs targeting mousep53 or human Bim were used as positive or negative controls, respectively. Excitingly, we were able to identify several genes accelerating Myc driven lymphomagenesis when compared to conventional Eμ-myc transgenic mice. Amongst known targets, we also identified novel tumour suppressor genes, which we are currently validating. In summary, we have established a quick and reliable whole mouse genome in vivo screening platforms allowing for the identification of novel tumour suppressor genes and hence potential drug targets for future therapies.

Improving HDR efficiency for CRISPR/Cas9 editing in mice.

Khin, Nay Chin, Lowe Jenna, Starrs Lora, Jing Gao, Nikki Ross and Burgio Gaetan.

Genome editing and Genetics of Host-Pathogens interaction: Department of Immunology and Infectious diseases. The John Curtin School of Medical Research, the Australian National University, Canberra, Australia.

Novel precision genetic technologies such as CRISPR/Cas9 genome editing technology offer novel avenues to a better understanding the mechanisms of diseases. Using CRISPR/Cas9 we are able to precisely modify the mouse or the human genome by creating knockout or a specific single nucleotide change to enable the study of the function of the gene of interest. The generation of these models lies on the ability of Cas9 to create a double strand break in the DNA and the repair to occur via the error prone Non-Homologous End Joining (NHEJ) or the precise Homology direct Repair (HDR) mechanisms. A large body of work has been recently dedicated to improve the technology to generate efficiently knockout or knock-in mouse models (point mutations, tags or floxed alleles). This presentation will give an overview on the factors that influence the likelihood of success to generate a point mutation or floxed allele in mouse or cell line using CRISPR/Cas9 genome editing.

Using CRISPR/Cas9 to identify novel drug targets for cancer therapy.

Brandon J Aubrey*[1,2,3], Gemma L Kelly*[1,2], Haoyu Yang*[1,2], Andrew Kueh[1,2], Margs S Brennan[1,2], Liam O'Connor[1,2], Liz Milla[1,2], Stephen Wilcox[1,2], Lin Tai[1], Andreas Strasser[1,2], and Marco J Herold[1,2]

[1.] Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, 3052 Australia, [2.] Department of Medical Biology, University of Melbourne, Parkville, Victoria 3050, Australia, [3.] Department of Clinical Haematology, The Royal Melbourne Hospital, Parkville, Victoria, 3050, Australia

*Contributed equally

CRISPR/Cas9 mediated genome engineering provides an easy method to modify genes *in vitro* and *in vivo*. However, most systems used so far showed low efficiency and were not broadly applicable in different cell types. In order to overcome this hurdle and to allow for efficient modification of genes in haematopoietic cells, we have developed a novel drug-inducible lentiviral CRISPR/Cas9 system. Using this system, we were able to validate the pro-survival BCL-2 family member MCL-1 as a critical survival factor in human Burkitt Lymphoma cells *in vitro* and *in vivo*. To identify other critical tumour suppressor genes, we performed a whole genome CRISPR/Cas9 knockout screen in Eμ-MYC lymphoma cells. ~30% of the top 30 candidate genes could be validated *in vitro.* In order to further validate some of the top candidates *in vivo*, we produced CRISPR knockout mice, which are currently analyzed. Taken together we have established a fast and reliable pipeline for the identification and validation of novel genes involved in tumour growth.

The application of CRISPR/Cas9 technology in generating complex mouse models

Andrew Kueh

The Walter and Eliza Hall Institute of Medical Research

The use of the CRISPR/Cas9 system in the one-cell stage embryo has revolutionized the speed, simplicity and efficiency in generating genetically modified mice. Although it is well accepted that simple modifications, such as gene deletions and small insertions are highly reproducible, the use of this methodology to develop mouse models with more complex genetic modifications, such as floxed or large knock-in alleles, has been associated with much lower success rates. Importantly, we note that the key reason for the low efficiency of CRISPR/Cas9-mediated generation of floxed alleles is due to the methodology outlined by the initial landmark studies describing the use of CRISPR/Cas9 in mouse zygotes. Here, we report that these limitations can be consistently and efficiently overcome by the use of large targeting vectors to introduce loxP sequences.

Predicting the HDR efficiency of CRISPR-Cas9

Aidan O'Brien[1], Nikki Ross[2], Jenna Lowe[2], Jing Gao[2], Nay-Chi Khin[2], Gaetan Burgio[2], Denis Bauer[1]

[1] CSIRO, Sydney, NSW, Australia, [2] Department of Immunology and Infectious diseases, The John Curtin School of Medical Research, The Australian National University.

The CRISPR-Cas system impacts many scientific fields, including reverse genetics, personalised therapies and cancer treatment. While the targeted knock-out of genes using the CRISPR-Cas9 system to cause indels has found many use cases, applying this system to insert or substitute genomic sequences promises an even larger application potential. Here, the homology directed repair pathway (HDR) repairs the double-strand break caused by the Cas9 enzyme by using a provided DNA template to integrate a precise change into the genome. However, the HDR pathway is the less frequently chosen pathway in mammalian cells, and the factors influencing pathway choice are currently not understood. We hence study a novel dataset that distinguishes sites repaired by HDR from sites where the cut was repaired by Non-Homologous End Joining (NHEJ) leading to indels. Using a Machine Learning approach, we identify sequence features that influence pathway choice and discuss the impact of template choice on the incorporation rate. From these insights, we develop the first CRISPR target site predictor to advice on HDR efficiency. The cross-validated accuracy is 0.6125. We find location specific sequence feature that promote HDR over NHEJ confirming that the seed region in addition to the template is an important modulator of activity. The resulting framework allows the user to design their CRISPR experiments around targets where HDR is more likely.

Crispr/Cas9 in in vivo modeling and drug discovery

Danilo Maddalo

Genetically modified mouse models have been key in the discovery and characterization of new targets in oncology. Despite their relevance, implementation of such models in the drug discovery pipeline has been limited by cost and time. In this talk we will discuss the impact the Crispr/cas9 is having on the drug discovery process and its applications.

Global cancer cohorts efforts

Sean Grimmond

The Vector and Genome Engineering Facility (VGEF): Customized service for your research needs

Predrag Kalajdzic[1], Kimberley Dilworth[1], Grober Baltazar Torres[1], Arcadius Rybicki[1], Ian E. Alexander[2], Patrick P.L. Tam[3] and Leszek Lisowski[1,4]

[1]Vector and Genome Engineering Facility, [2]Gene Therapy Research Unit, [3]Embryology Research Unit and [4]Translational Vectorology Research Group, Children's Medical Research Institute, University of Sydney, 214 Hawkesbury Road, Westmead NSW 2145, Australia

The mission of the Vector and Genome Engineering Facility (VGEF), a recently established research core of the Children's Medical Research Institute, is to support basic and translational research in vector-based gene therapy and genome engineering. In addition to being a service facility that provides investigators the access to the latest vector and genome engineering technologies, VGEF is also active in developing new, and improving existing, technologies that will empower its clientele and the wider scientific community. The VGEF facility offers customized preparations of lentiviral (LV), adenoviral (Ad), and adeno-associated viral (AAV) vectors, at purities and production scales tailored to the needs of individual projects to facilitate both in vitro and in vivo experimentation, as well as a range of ready-to-use AAV and LV stocks. In addition to vector-based tools, VGEF offers genome-editing services in mouse and human cell lines, mouse ES cells and human iPS cells. VGEF closely follows and utilizes the latest advances in genome editing technologies. Currently VGEF uses a multitude of CRISPR/Cas9-based systems, such as custom Cas9 nucleases for precise genome modifications, and endonuclease-free AAV-mediated editing. In alignment with our goal to promote vector-based and genome engineering technologies, we offer consultation in study design and technical support in the selection of appropriate tools for specific experimental needs, as well as hands-on training opportunities. Beyond our academic and commercial services, we also engage in collaborative studies that are aligned with our research focus in AAV biology, vector development and genome engineering.

Using of gene editing to treat cancers both in vitro and in vivo

Nigel McMillian

Using machine learning to understand CRISPR-Cas9 activity

Laurence Wilson[1], Aidan O'Brian[1], Robert Dunne[2], Oscar Luo[1], Denis Bauer[1]

[1] CSIRO, Sydney, NSW, Australia [2] CSIRO, Data 61, North Ryde Sydney

The CRISPR-Cas9 system is one of the most widely utilized genome editing mechanism, allowing the precise targeting of specific genomic loci and has the potential for application in human health. However, the reliable application of the technology requires the identification of the optimal target site as activity can vary substantially. Choice of the optimal target site requires balancing efficiency (on-target activity) and specificity (minimal off-target effects).

We have utilized machine learning to develop models to predict target efficiency and specificity. On-target efficiency was modelled using a training dataset of over 1000 sgRNAs across two cell lines, allowing us to identify cell-specific factors in the chromatin environment that contribute to activity. Our final model combines both sequence features and histone modifications to accurately predict target efficiency, outperforming other currently available models. Our target specificity pipeline utilizes a new bi-directional mapper which allows it to rapidly identify potential off-target sites based on sequence similarity. Importantly, our pipeline is the first of its kind to incorporate variant information, enabling it to identify off-target sites unique to a given individual or population. We have implemented our models as a free web-app utilizing new developments in serverless computing from AWS, making it a highly scalable and efficient tool for CRISPR-Cas9 target design.

Yeast 2.0: Design, synthesis, and testing of a Eukaryotic genome

Hugh Goold

What to expect from your CRIPSR experiment: Finding downstream effects adjacent to your primary target

James D Doecke

CSIRO Health and Biosecurity/Australian e-Health Research Centre, Brisbane, QLD Australia

The aim of using CRISPR in clinical trials revolves around treating human diseases via modification of pathology causing variants to reinstate the homeostatic process, and remove the impetus for further disease modifying pathologies. To do this though, care needs to be taken to measure adjacent or downstream effects from genomic modification in animal models. Whilst the immediate effects to the DNA of single nucleotide changes are implicit, it is hard to estimate what the indirect downstream effects to the mRNA and protein might be. It is possible that a single modification could prematurely terminate protein synthesis resulting in an altered peptide yield in the protein. Since most transcripts are either highly regulated or are potent regulators themselves as part of multiple networks, it bides that a single modification maybe responsible for many effects to multiple networks. Thus analyses of all networks involving the modified sequence should be investigated.

Genome wide screens to develop new targets for cancer therapy

Amee J. George[1], Priscilla Soo[1] , Nadine Hein[1], Maurits Evers[1], Lorey K. Smith[2], Mei S. Wong[1,2], Lorena Nunez[1], Kaylene J. Simpson[3], Richard B. Pearson[2], Katherine M. Hannan[1] and Ross D. Hannan[1,2]

[1] ACRF Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, Australian National University, Acton 2601 Australia; [2] Oncogenic Signalling and Growth Control Program, Peter MacCallum Cancer Centre, Victorian Comprehensive Cancer Centre, Melbourne 3000, Australia;

A major therapeutic mechanism of action of cytotoxic drugs is the induction of p53, a key tumour suppressor protein that restrains tumour cell growth by inducing cell cycle arrest, senescence or cell death. Recent studies have revealed that p53 is also rapidly and potently induced by non-genotoxic approaches, such as disruption of the nucleolus following perturbation of ribosome biogenesis, the primary function of the nucleolus. Indeed, it appears that monitoring and maintaining ribosome biogenesis and nucleolar integrity (termed the nucleolar surveillance pathway) is critical for regulation of p53 activity in normal cells and suggests that selective nucleolar disruption provides a new mechanism for the activation of p53 to kill cancer cells (Bywater et al Cancer Cell 2012; Devlin et al Cancer Discovery 2016; Hein et al Blood 2017). This idea is now being tested in humans with two current Phase1/b clinical trials of selective inhibitor of Pol I transcription, CX-5461 that we have developed. To better understand the role of nucleolar stress activation in cancer, we recently performed a genome-wide screen RNAi screens to ascertain which genes modulate p53 stabilisation via the nucleolar surveillance pathway. We will present our results of these screens which surprisingly demonstrate that the nucleolar surveillance pathway is essential for the robust stabilisation of p53 in response to most, if not all forms of cellular stress. Our studies have important implications for the regulation of p53 in malignant transformation and place the nucleolus as the central cellular regulator of this important tumour suppressor.

Gene-editing in immune checkpoint discovery

Jai Rautela

The Walter and Eliza Hall Institute of Medical Research

The description of 'a balance of activating and inhibitory signals' is a necessarily vague understanding of how an NK cell makes the decision to kill a target cell. From this, it appeared that a complex immunotherapeutic strategy would likely be required to improve the anti-tumor function of NK cells. By performing a combination of genetic and small molecule screens we have begun to unravel the major druggable pathways the regulate NK cell function. Our recent discovery of a novel cytokine-induced checkpoint ('CIS') has highlighted the efficacy of these approaches, and suggested that there may, in fact, be relatively few unifying pathways that we can target to improve endogenous or adoptive NK cell function. Using CRISPR Cas9 we now study NK cell biology by making gene-edited humanized mice and validate these findings by genetically manipulating primary human NK cells. We will discuss how is CRISPR is fast becoming a key tool in understanding how the immune system interacts with a cancer, and how its leading the discovery of new immunotherapeutic targets.

A whole genome CRISPR/Cas9 screen for regulators of JAK-STAT signaling reveals epigenetic control by Setdb1

Andrew Jarratt [1], Adrienne Hilton [1], Ladina Di Rago [1], Alexandra L Garnham [1], Kylie Greig [1], Warren S Alexander [1], Nicos A Nicola [1], Douglas J Hilton [1]

[1.] Walter and Eliza Hall Institute, Parkville, VICTORIA, Australia

Activation of the JAK-STAT signaling pathway by interleukin 6 (Il6) is required for multiple cellular immune and inflammatory responses, including macrophage activation and the repair of damaged tissue. Binding of Il6 to Il6 receptor alpha (Il6ra) initiates signaling through the transmembrane gp130 protein leading to activation of the JAK-STAT signaling cascade and, ultimately, changes in expression of STAT target genes. To date the functions of the proteins within the pathway have been well characterized, but it remains possible that other regulators exist. To screen for novel regulators of the JAK-STAT pathway, we used a Cas9-expressing M1 murine leukaemia cell line, infected with the GeCKO whole genome gRNA library. M1 cells form tight, clonal colonies of cells when plated in semi solid agar, which disperse and differentiate into macrophages when treated with Il6. Factors that perturb the JAK-STAT pathway result in unresponsiveness to Il6 treatment and cells fail to differentiate, thus providing a clear readout for screening experiments. Unresponsive clones can then be expanded and sequenced to identify gRNAs that may be driving the phenotype. In our whole genome screen, one Il6 unresponsive M1 clone contained a gRNA targeting the H3K9 methyltransferase, Setdb1. Subsequent infection of M1-Cas9 cells with six gRNAs targeting Setdb1 resulted in clonal lines that were all hypo-responsive to Il6 stimulation, even at ten times the standard concentration. As Setdb1 is involved in gene silencing via tri-methylation of H3K9 tails, we have carried out RNA-Seq to identify differentially expressed genes when comparing the Setdb1 knockout lines to the M1-Cas9 line. In addition, we have performed ChIP-Seq to look for altered H3K9Me3 at genes in the knockout vs. M1-Cas9 cells. Combining these data sets, we aim to identify the Setdb1-regulated gene or gene network critical for this new level of control of the Il6 activated JAK-STAT signaling pathway.

Investigating the Resistant Mechanisms to the BCL2 selective inhibitor Venetoclax in Multiple Myeloma using Genome-Scale CRISPR/Cas9 Knockout Screening

Jia-Nan Gong[1,2], Mark F van Delft[1,2], Marco J Herold[1,2], Andrew W Roberts[1,2,3], David CS Huang[1,2]

[1] The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
[2] Department of Medical Biology, University of Melbourne, Melbourne, Australia
[3] The Royal Melbourne Hospital, Melbourne, Australia

Multiple myeloma is a clonal B-cell malignancy characterized by complex genetic aberrations. In spite of recent improvements, this disease remains incurable and treatment options for relapse/refractory disease are limited. A promising class of novel agents to treat patients with other B-cell malignancies are the BH3 mimetic compounds to target the pro-survival BCL2 proteins. Remarkably, ~80% of patients with refractory or relapsed chronic lymphocytic leukaemia in clinical trials respond to venetoclax, a BCL2 selective inhibitor. We set out to determine the identity of pro-survival BCL2 protein responsible for the survival of myeloma cells.

To address this, we firstly tested the response of 31 myeloma cell lines to the BCL2 selective inhibitor venetoclax. Interestingly, about a quarter of myeloma cell lines was readily killed when BCL2 was inhibited by venetoclax. The dependence of myeloma cells on BCL2 for their survival was further validated using CRISPR/Cas9 and cells were rapidly killed in the absence of BCL2. Encouragingly, early-phase studies have recently shown that inhibition of BCL2 holds promise for some patients with myeloma, particularly those with t (11;14). We are therefore very keen to understand why some myeloma cells respond well to venetoclax and how they might acquire resistance to the treatment. To do this, we generated the venetoclax-resistant sub-lines using KMS-12-PE myeloma cell line, which is highly sensitive to BCL2 inhibition. Interestingly, MCL1 protein level was elevated in all the resistant sub-lines. We are now setting up the genome-scale CRISPR/Cas9 knockout (GeCKO) screening to look further into the resistant mechanisms using myeloma cell lines, in anticipation that our findings are likely to be highly relevant to patients with multiple myeloma. Taken together, our mechanistic studies strongly suggested that inhibiting the activity of MCL1 is likely to enhance the response of myeloma cells to BCL2 inhibition, provided the on-target toxicity of this combination is tolerable and can be managed.

CRISPR/Cas9 screens reveal VDAC2 to be a critical regulator of BAX-mediated cell death and chemotherapeutic response

Hui San Chin*[1,2] , Mark F van Delft*[1,2] , Mark X Li[1,2] , Kirsten Scicluna[1,2] ,
Iris KL Tan[1] , Gemma L Kelly[1,2] , Stephane Chappaz[1,2] , Seong L Khaw[1,2] ,
Marco J Herold[1,2] , Daniel H Gray[1,2] , David CS Huang#[1,2] , Grant Dewson#[1,2]

[1] Walter and Eliza Hall Institute, Parkville, Australia,
[2] University of Melbourne, Department of Medical Biology, Parkville, Australia

The BCL-2 family proteins BAX and BAK are critical effectors of the intrinsic pathway of apoptosis. Using genome-wide Crispr/Cas9 genetic screens we identified voltage-dependent anion channel 2 (VDAC2), an anion channel that transports hydrophilic solutes across the mitochondrial outer membrane, as essential for BAX to mediate apoptosis. Deletion of VDAC2 in tumour cells reliant on BAX for apoptosis abrogated their response to BH3-mimetic drugs and conventional chemotherapeutic agents both in vitro and in vivo . Deletion of Vdac2 also accelerated tumourigenesis in a c-MYCdriven model of acute myeloid leukaemia. Vdac2-/- mice, generated on a C57BL/6 background through CRISPR/Cas9 gene targeting, were viable, which is in contrast to the previously reported lethality of Vdac2-/- embryos on a mixed genetic background. Vdac2-/- mice exhibited no evidence of excessive BAK-mediated apoptosis, challenging the notion that the principal role of VDAC2 in apoptosis is to inhibit BAK. Our data re-defines the role of VDAC2 in apoptosis and identifies a critical interaction between BAX and VDAC2 that influences tumour development and chemotherapeutic response.

#,* These authors contributed equally

CRISPR: Innovation and commercialisation in a complex IP environment

Sally J. Davis

Patent Attorney
Davies Collison Cave Pty Ltd

CRISPR-mediated gene editing is widely considered to be one of the seminal scientific discoveries of the century. The commercial application of the technology for the development of new agricultural, therapeutic and industrial biotechnology innovations has resulted in large-scale conflicts between the pioneers of CRISPR-mediated gene editing as they scramble to establish patent rights over the technology. While some recent legal challenges to key US patent applications covering the initial CRISPR-Cas9 systems discovered by Doudna and Charpentier are yet to be fully resolved, this has not prevented the grant of other patents protecting the technology across the world.

In view of the complex web of patent rights around CRISPR, it is important for researchers to recognise the impact of using CRISPR for research and/or commercial purposes. Furthermore, the commercialisation of any down-stream innovations that develop, extend or apply the CRISPR technology may require authorisation from those that hold patent rights in this space. In this presentation, the current Australian CRISPR patent landscape will be outlined, with a specific focus on the rights held by Doudna, Charpentier and Zhang. Thereafter, the implications of patent rights existing in this space will be set out, inclusive of how these rights may be infringed. Finally, strategies for ensuring freedom to operate will be discussed.

CRISPR/Cas9 and bioethics

Rachel Ankeny

Popular media and scientific fora are abuzz with news of novel techniques for genomic editing for various purposes including altering genes to prevent human disease particularly for single gene disorders, modifications of non-human organisms to develop better animal models for some diseases, and more generally for developing deeper understanding of the structure, function, and regulation of genes. Despite numerous calls for engaging the public before these techniques become even more well-established, such efforts have been limited to date. This paper explores a range of ethical issues associated with genomic editing techniques in concert with an analysis of recent media coverage on these technologies, in order to underscore the need to focus on the potential value conflicts underlying use of these technologies and to anticipate public reaction in a thoughtful and productive manner, unlike what arguably occurred in the case of debates over genetic modification and other emerging technologies.

Advancing genome editing technology adoption with computational innovations.

Chang Yu

The utility of genome editing technologies for disease modeling and developing cellular therapies has been extensively documented however the impact of these technologies on mutational load at the whole-genome level remains unclear. We could perform whole-genome sequencing to evaluate the mutational load at single-base resolution in individual gene-corrected, and with careful monitoring via whole-genome sequencing it is possible to apply genome editing to human cells with minimal impact on genomic mutational load.

Since doing so, NGS instruments have generated petabytes of data and the rate of data being generated has surpassed that of Moore's Law, with vast increases in the scale and scope of genome sequencing projects. Furthermore, the use of sequencing technology has become a standard practice of clinicians and researchers. To achieve this, computational cycles and storage capacity can be leased from highly integrated solutions based bioinformatics tool suites. This lowers the entrance barrier for working with sequencing datasets increasing the adoption of genomic technologies.

To overcome these challenges, researchers have been utilizing IT technologies with leading life science institutes in China, BGI and Novogene to achieve better results. These solutions being used by researchers that are increasing the rate at which genome editing is developed and lowering innovation barriers.

CRISPR-Cas9 knockout screening identifies novel anti-cancer drug resistant genes

Raymond Man Tat Lau[1], G. Gregory Neely[1]

[1] Dr. John and Anne Chong Lab for Functional Genomics, Charles Perkins Centre and School of Life and Environmental Sciences, University of Sydney, Camperdown, NSW 2006, Australia

Cancer drug resistance is the most common cause of death in cancer patients. An understanding of the drug mechanism of action and identification of accurate resistance biomarkers is required to develop rational drug and personalized combinations. We have performed genome-wide lenti-CRISPR knockout screens to isolate genes involved in drug resistance for a panel of 29 anti-cancer drugs under clinical and preclinical investigation. Our efforts have identified a collection of known and novel genes whose loss-of-function mutations lead to cancer drug resistance. By comparing the molecular fingerprint of drug resistance for these anti-cancer drugs, and by classifying various drug resistance mechanisms at the molecular level, our work can have an important impact on new drug development and the design of the most effective combinational treatment strategies to better treat cancer patients.

The inexplicable tumour suppressor behaviour of FUBP1 in Oligodendroglioma.

Olga Zaysteva[1], Naomi C Mitchell[1], Linna Guo[1], Ross D Hannan[1,3], Mark R Gilbert[4], David L Levens[4], Leonie M Quinn[1,2]

[1] Department of Cancer Biology and Therapeutics, John Curtin School of Medical Research, ANU, Acton, Canberra, AUSTRALIA. [3] Peter MacCallum Cancer Centre, Melbourne, AUSTRALIA. [4] Center for Cancer Research, NIH, Bethesda MD, USA.

The best-case scenario for Oligodendroglioma patients is a prolonged disease (>15 years) associated with significant morbidity (including seizures and neurological deficits). More alarming are recent observations that standard of care therapies can actually cause a subset of these oligodendrial tumours (around 15%) to become extremely aggressive to rapidly cause mortality. The reasons for this are unclear, but current histopathological classification methods do not reliably predict response to treatment. Thus, new molecular prognostic markers to personalise treatments are imperative if we are to increase outcomes for Oligodendroglioma patients.

FUBP1 ranks in the top 10% of predicted driver mutations in primary Oligodendrial brain cancers (The Cancer Genome Atlas (TCGA)). However, Oligodendroglioma genetics is complex, with several frequently identified driver mutations (e.g. *CIC*, *NOTCH1*, *PIK3R1*/*PI3KCA, AGO1, EPC2*, *HDAC2*) co-occurring with *FUBP1*. As the consequence of these mutant combinations to tumour behaviour is unknown, we have set out to determine how specific Oligodendroglioma genotypes correlate with tumour progression. To clearly connect genotype and phenotype, we are developing genetically tractable *in vivo Drosophila* and mouse models, using both RNAi and CRISPR. Thus, we can determine if *FUBP1* and/or the network of co-occurring mutations identified in Oligodendroglioma, alter glial lineage behaviour in the context of an otherwise normal signalling environment. Further determining if the mutations identified by genome-wide studies might provide prognostic markers to guide treatment using current drug therapies, we are working to determine efficacy of small molecules targeting the FUBP-axis. Thus, we will open new avenues for glioma treatment to ultimately improve patient outcomes.

Enhanced, streamlined approaches to facilitate CRISPR-mediated gene activation

Ben Hayley

CRISPR/Cas9 is conventionally used as a loss-of-function tool within the context of cell-based genetic screens. However, modification of Cas9 can convert it from a targeted DNA damaging enzyme into a transcriptional activator, thus enabling its use for gain-of-function studies. We have developed a suite of vectors that streamline the process of generating a stable CRISPR/Cas9 activation-competent cell line. Here, we will describe the comparative performance of different Cas9-activator combinations expressed from these vectors, and we will discuss best practices and potential pitfalls when engineering cells to be competent for CRISPR-mediated gene activation.

Editing the epigenome

Ryan Lister

Australian Research Council Centre of Excellence in Plant Energy Biology, Harry Perkins Institute of Medical Research, The University of Western Australia, 35 Stirling Hwy, Crawley, WA 6009, Australia

Covalent modifications of DNA and histones play critical roles in the regulation of gene expression, cell activity, development, and disease. DNA methylation is a critical layer of the vertebrate epigenome, however despite several decades of investigation, the precise roles of DNA methylation in the control of genome and cell activity are still not clearly understood. A major obstacle in deciphering the mechanistic roles of epigenomic modifications has been the inability to precisely control and change the modification states in the genome. However, genome editing technologies are now rapidly being repurposed to achieve editing of epigenomic modifications where desired in the genome, in order to elucidate the causal relationships between these modifications and genome regulation, and as artificial regulatory tools to control cell activity and identity. We employed a broadly active artificial epigenome modifying protein to achieve genome-wide manipulation of promoter DNA methylation, enabling comprehensive assessment of its effects upon transcription and histone modifications, and the stability of artificially induced methylation. Furthermore, we have developed new CRISPR-Cas9 based tools that enable highly specific addition or removal of DNA methylation at desired locations in the genome in a controlled fashion. In addition to optimizing the efficacy and specificity of these functional epigenomics tools, we have utilized them to explore the sensitivity of DNA binding proteins to DNA methylation state. Overall, recent developments in epigenome editing tools are providing new insights into the role of covalent genome modifications in regulating gene expression, and new platforms for the manipulation of cell activity and identity.

High throughput CRISPR screening approaches

Iva Nikolic, Karla Cowley, Jennii Luu, Robert Vary, Nathan Crouch, Kaylene Simpson

Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre, Vic

Discovery-based genome-scale screening has been the mainstay of the VCFG for the past 9 years. Using the expertise developed using RNA interference screening approaches, the lab has now developed high throughput CRISPR screening, both in terms of pooled viral approaches and arrayed synthetic screens. This talk will cover examples of both screening approaches, highlight scenarios where different approaches work best and provide a detailed insight into quantitative phenotypic screening using high content imaging.

Integrative Big Data Models for Precision Oncology

Veera Baladandayuthapani

MD Anderson Cancer Center, Houston, Texas.

Modern biomedicine has generated unprecedented amounts of data. A combination of clinical, environmental and public health information, proliferation of associated genomic information, and increasingly complex digital information have created unique challenges in assimilating, organizing, analyzing and interpreting such structured as well as unstructured data. Each of these distinct data types provides a different, partly independent and complementary, high-resolution view of various biological processes. Modeling and inference in such studies is challenging, not only due to high dimensionality, but also due to presence of structured dependencies (e.g. pathway/regulatory mechanisms, serial and spatial correlations etc.). Integrative analyses of these multi-domain data combined with patients' clinical outcomes can help us understand the complex biological processes that characterize a disease, as well as how these processes relate to the eventual progression and development of a disease. This talk will cover statistical and computational frameworks that acknowledge and exploit these inherent complex structural relationships for both biomarker discovery and clinical prediction to aid translational medicine. The approaches will be illustrated using several case examples in oncology.

Extracting the most out of your multi-omics data.

Jean Yang

Faculty of Science, University of Sydney, Sydney NSW, Australia

With the advancement of many high-throughput biotechnologies, an interest of many researchers has been to utilize multiple high-throughput data sources to gain further insights into biology and obtain a deeper understanding of complex diseases. Integration enables scientist to address and ask very specific questions utilizing significant testing frameworks. An example includes the joint use of microRNA (miRNA) and mRNA platforms to identify if a miRNA is differentially expressed and if its predicted targets, which lie in a common biological pathway, have changed in the opposite direction. More commonly, integration enables us to explore and understand the complex relationships among different molecular phenotypes. I will discuss how rapidly emerging tools in network research allows us to explore dynamic and static mutation-expression networks through combining exome sequencing and mRNA data. Finally, the modeling of heterogeneity in multi-omics data together with extracting different types of features can help to improve the prediction of prognosis and disease outcome. In this talk, I will discuss briefly the different ways to interrogate multi-omics data and the different types of questions we have addressed.